STUDIES ON UNSTABLE CHARACTERS IN PETUNIA.

I. THE EXTREME FLOWER TYPES OF THE UNSTABLE RACE WITH MOSAIC COLOR PATTERNS

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INTRODUCTION

Since the publication of DE VRIES' "Mutationstheorie" numerous cases of unstable characters have been examined. The first account of the inheritance of the striking somatic variations so commonly found in variegated plants was given by DE VRIES (1901) in his discussion of eversporting varieties. At the present day thanks to the results obtained by EMERSON (1917), EYSTER (1928), DEMEREC (1928, 1931), OEHLKERS (1930) and others the explanation of the behavior of unstable characters on the basis of the theory of unstable (that is, frequently mutating or "mutable") genes seems to be the most probable.

The unstable characters observed in plants concern mostly the distribution of pigmentation in leaves, flowers and other plant organs. In the unstable race of *Petunia violacea* Lindl. we have to deal with a large range of variation not only with regard to the color patterns on petals but also with regard to other characters both physiological and morphological. All those characters seem to be determined by one unstable gene with pleiotropic effect.

The extreme types of the unstable race of *Petunia violacea* differ in the following characters:

1. in the sizes of flowers
2. in the distribution and the amount of pigment in the flowers
3. in the sizes of the epidermal cells of petals
4. in the percent of abortive pollen grains
5. in the percent of germinating seeds.

Between the extremes are many gradations regarding all just-mentioned characters. The larger the flowers are the more pigment they contain, that is, the more numerous are the patches and the more densely they are distributed on the surface of the petals. Corresponding to the increase of the sizes of flowers the percent of germinating seeds increases and the number of abortive pollen grains becomes less numerous. Each flower type may reproduce the whole range of variation characteristic of the unstable race but it gives a majority of plants like itself, or in neighbouring classes of mosaic, with relatively fewer in classes remote from parental type. The selection of the extreme flower types results in the isolation of particular lines differing from one another in the frequency of mutation.

The variability of the unstable race reveals itself in the occurrence of different flower types not only in different plants but also in one individual (sectorial flowers and sectorial plants). The variability of particular shoots may also be a continuous one when the flowers of related types succeed one another. The experiments on crossing of the unstable race with the variety "Admiration" (belonging to *P. violacea*) show that the mosaic distribution of pigment is a recessive character, the uniform distribution being a dominant one. These characters are transmitted independently of the violet color, the sizes of flowers and the distribution of pigment in the form of stripes characteristic of the variety "Admiration". The unstable race has been also crossed with white *Petunia nyctaginiflora* and the F_1 plants have had also self-colored flowers. In this paper I give a detailed description of the two extreme types of plants of the unstable race leaving to further publications the presentation of the data concerning the selection experiments as well as those regarding the behavior of chimeral plants.

THE FLOWER TYPES

The differences in sizes between the extreme flower types are marked. The smallest flowers have about 1.5 cm in diameter while the largest ones about 5.0 cm. Some flowers of the unstable race are shown in figure 1. We see the extreme types (figure 1A and figure 1F) and some intermediate forms (figure 1B-E). The amount of purple pigment varies with the sizes of flowers. In small flowers it is distributed in the form of spots and patches in the epidermis while the large flowers are self-colored. The larger the flower, the larger on an average are also the purple patches. The correlation between the amount of pigment and the sizes of flowers is a significant one and the coefficient of correlation equals 0.837 (MALINOWSKI and SMÓLSKA 1932). The smallest spots on the petals of mosaic flowers have dilute purple color, the larger ones being always darker. In the small flowers the purple spots appear chiefly at the midrib of the flower lobes. In larger ones the middle part of the petals is self-colored, the margins being spotted and patched. As I have pointed out, the sizes of spots are proportionate to the sizes of petals. In larger petals they fuse with one another and between large patches so produced narrow and irregular spaces of white or light purple color are seen. The narrowest spaces are of light purple color, the broader ones being white. Figures 2 and 3 show the distribution of white, purple and light purple areas on small, intermediate and large petals. In these figures we see the drawings representing the halves of flower lobes with exact distribution of purple and light purple spots. The purple spots are shown in black, the light purple ones in grey.

The anthocyanin is confined to the epidermal cells of flowers.

ANATOMICAL RESEARCHES

The epidermis of petals on both the upper and the lower surface is composed of larger cells in large self-purple flowers than in small white ones. In mosaic flowers the epidermis of white area is composed of smaller cells than that of purple area. The epidermal cells of purple area and those of
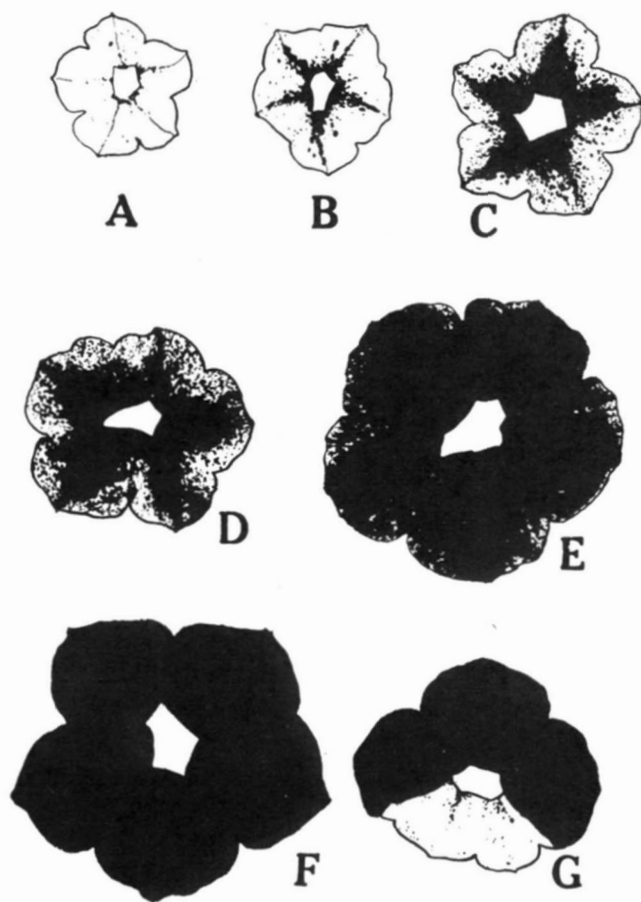

FIGURE 1.—Seven flowers of the unstable race. A and F—the extreme types; B, C, D, E—
intermediate forms; G—sectorial chimera. Natural sizes.

the self-purple flowers are of papillose type as far as the epidermis of the upper surface is concerned. On the lower surface of petals in both extreme types of flowers the epidermis is composed of cells with irregularly lobed outline (figure 4). Besides this the cell walls of the lower surface form folds or "loops" directed to the inside of the cell. Such loops may also be observed in the upper epidermis of the self-purple flowers but in this case they are much smaller and in our preparations (figure 4) they look like

minute protuberances. In the upper epidermis of the white areas of the mosaic flowers the protuberances are missing.
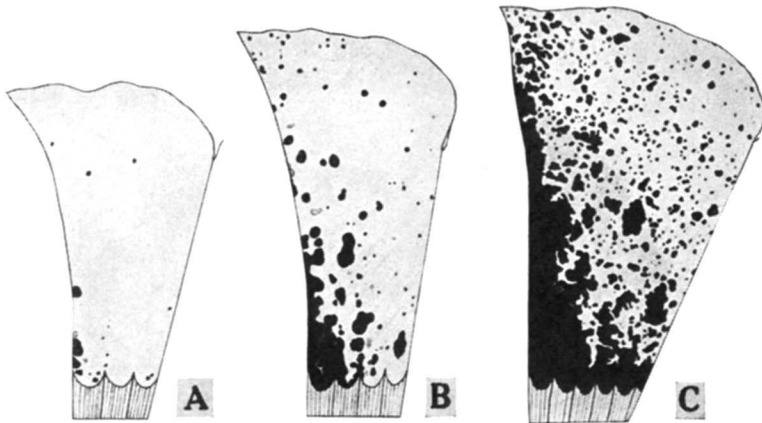

FIGURE 2.—Halves of petals of three flowers shown in figure 1, denoted by the letters A, B, C. The letters in both figures (i.e. figures 1 and 2) refer to the same flowers. Dark purple spots are shown in black, light purple ones—in grey.
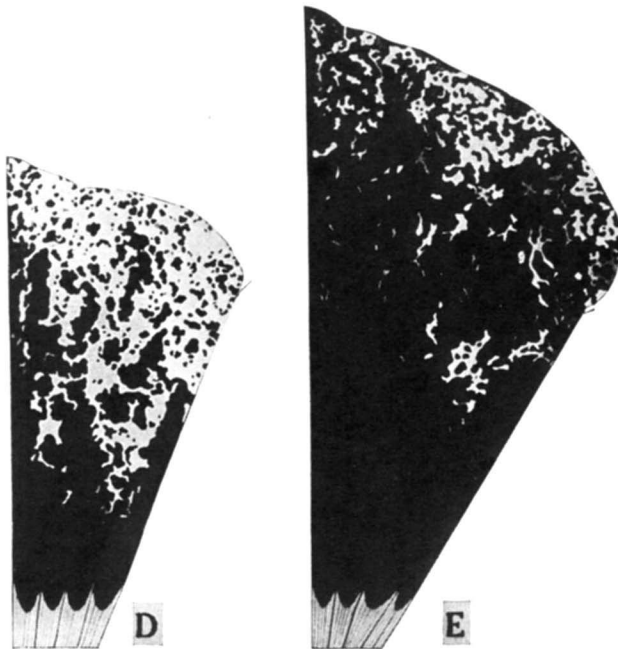

FIGURE 3.—Halves of petals of the flowers shown in figure 1 and marked by the letters D, E. The letters in both figures (i.e. figures 1 and 3) refer to the same flowers. Dark purple spots are shown in black, light purple ones in grey.

Let us pass now to the anatomy of small purple spots of the mosaic flowers. In figure 5c, a spot is shown composed of 8 purple cells. Their

walls possess small protuberances. This group of 8 cells is surrounded by smaller and distinctly lighter ones. The walls of these cells have not any
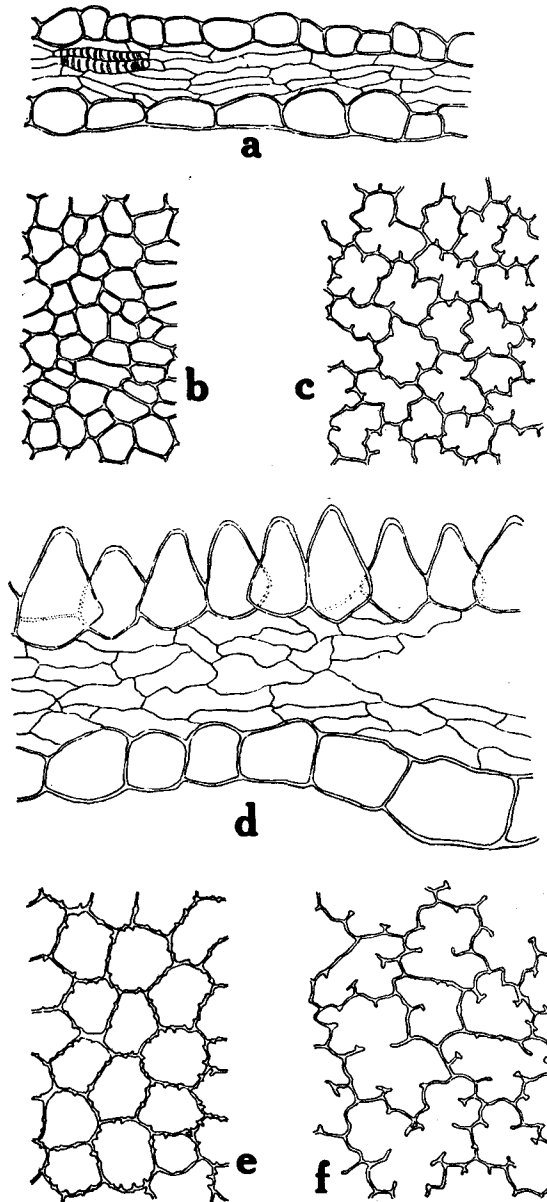

FIGURE 4.—Anatomy of petals of two extreme flower types. a, b, c—cross section (a) and the upper (b) and lower (c) epidermal cells of a small mosaic flower; d, e, f—cross section (d) and the upper (e) and lower (f) epidermal cells of a large self-purple flower.

protuberances. There are one or two layers (sometimes more) of such intermediate cells and around these layers we find small white cells char-

acteristic of the white areas of petals. Every purple spot either small or large is surrounded by a layer of intermediate cells. Such cells are distinctly lighter and a little smaller than the purple ones. In the flowers with white areas confined to small irregular spaces we also find intermediate cell layers. The border line between purple and white tissue is never sharp.
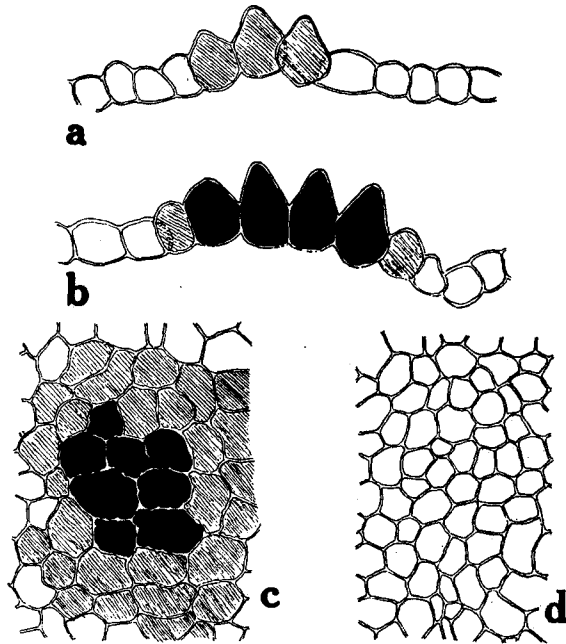

FIGURE 5.—Anatomy of a mosaic flower. a—three cells (shaded) of a light purple spot; b—four cells (represented in black) of a dark purple spot; c—a dark purple spot seen from above (dark purple cells shown in black, light purple ones are shaded); d—tissue of the white area of the petal.

Figure 6b shows a cross section of a chimeral petal. We see from this drawing that the upper epidermis of the purple area is composed of larger cells than that of the white area. The border line in this particular case is sharper than we have seen formerly between the purple spots and the surrounding white tissue. In this cross-section we see only one intermediate cell and its color is a little darker than that observed usually in the intermediate cells surrounding the dark purple spots.

DEMEREC (1931), who observed in Delphinium small purple spots surrounded by distinctly lighter cell layers, did not however find any differences in sizes between the dark purple cells in the center of the spot and the lighter purple cells of the surrounding layer. From the drawings given by this author we see that the white cells are of the same size as the dark purple and the lighter purple ones.

In Petunia the cells of the white area are much smaller than the colored cells in the center of the purple spot. The light purple cells are intermediate as to their sizes between the dark purple and the white ones.

DEMEREC assumes that the dark purple and the lighter purple cells differ genetically, namely, that the dark purple cells only have the reverted purple allele while the light purple color of the cells on the border line of purple spots is produced by some substance which diffuses from the dark purple cells into the adjacent pink cells. But the question arises whether the plant pigments can diffuse from one cell into the other. KRENKE (1933, p. 514) summarizing the results of investigations of many
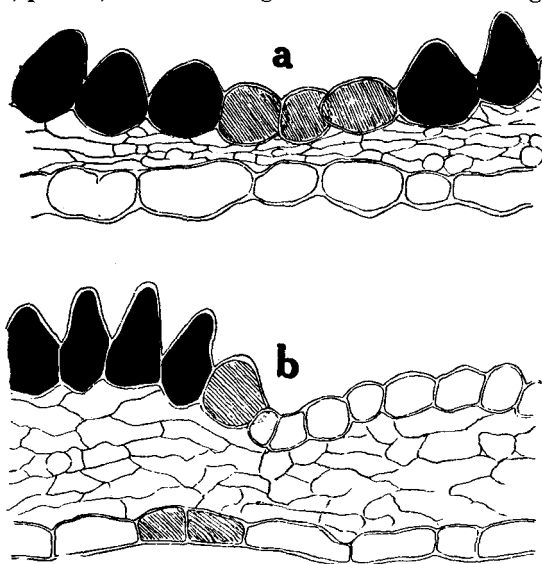

FIGURE 6.—a—cross section of the light purple cells surrounded by dark purple tissue; b—cross section of a chimeral petal of the flower shown in figure 1 G.

authors on the possibility of the passing of plant pigments from one cell into another writes as follows: "Die Farbstoffe der Pflanzen sind entweder in Protoplasma (in den Plastiden) oder aber in Zellsaft eingeschlossen. Ohne Zellverletzung (Abtötung oder Zerreissung) kommen diese Stoffe nicht einmal in die Nachbarzelle".

As I have pointed out there exists a correlation in Petunia flowers between the sizes of cells and the degree of coloration of their sap. The same causes therefore which produce lighter purple color probably determine also the smaller cell sizes.

In the smallest colored spots all cells are of a lighter purple color (the dark purple cells are missing). Such cells are also smaller than the dark purple ones characteristic of larger spots. It would be difficult to suppose that in this case the substance producing the lighter purple color of those

cells diffuses from the dark purple cells because such cells do not occur at all in the spots in question.

When two large spots are lying near each other the cells between those spots are of a lighter purple color and their sizes are a little smaller than those of the dark purple cells. Their walls are also deprived of small protuberances characteristic of the dark purple cells. Such cells are shown in cross-section in figure 6a. We see from this figure that they are smaller than the adjacent dark purple cells. Purple cells are present also on the lower surface of the petals. Here the anthocyanin pigmentation is distributed in patches or stripes running more or less parallel to the midrib of the petal. The anthocyanin distribution on the upper surface of the petal does not correspond usually to that of the lower surface.

DEMEREC assumes that each small purple spot in Delphinium is a result of mutation. "The mutations", writes this author, "occurring in the epidermal cells of sepals and petals show up as purple spots". The sizes of the spots of changed tissue, according to DEMEREC, can be used as an indication of the time when the mutation occurred, large spots being the early mutations and small spots being the late ones. I do not think we can explain in such a way the occurrence of purple spots on the petals of Petunia. Some arguments against such a supposition were quoted above. Some others will be published in another paper. It seems more probable that the mutable alleles determine different types of anthocyanin distribution shown in figure 1. Some of those alleles may produce a few small purple spots, the others may be responsible for a larger number of large purple spots. These alleles are of pleiotropic nature and they also influence the sizes of flowers, the degree of pollen sterility, the percent of germinating seeds and so on. All these characters are strongly correlated in our unstable race of Petunia.

It is important to note that in the flowers of Petunia not only the epidermis derives from dermatogen. Dr. H. TELEŻYŃSKI found in the cytological preparations of the normal diploid race of P. violacea one very young flower bud showing periclinal arrangement of diploid and tetraploid tissues The tetraploid cells were found in the epidermis of this bud and also in the tissue of the petal margins, underlying the epidermis. We must assume that this tetraploid tissue originated through mutation from a diploid cell of the dermatogen layer. It would be difficult to imagine other causes which could produce in this case such a periclinal chimera. The epidermis of both the upper and the lower surface of the corolla is composed of tetraploid cells and besides the tetraploid tissue was found in the inner part of the margins of all flower lobes. I think we may safely assume that in the buds composed of diploid cells only the border line between dermatogen and periblem is the same as in those with tetrapolid dermatogen. During

the growth of flower buds the margins widen more intensively in large self-purple flowers than in small mosaic ones. Thus the differences in sizes of flowers as well as those concerning the amount of pigment are associated with the tissue derived from dermatogen.

The genes acting in the dermatogen layer produce simultaneously the definite sizes of petals and the definite amount of pigment in the epidermis. Somatic mutations occurring very often in the unstable race of Petunia and resulting in sectorial chimeras always concern both characters simultaneously. Of course the genetic constitution of the inner cell regions, de-
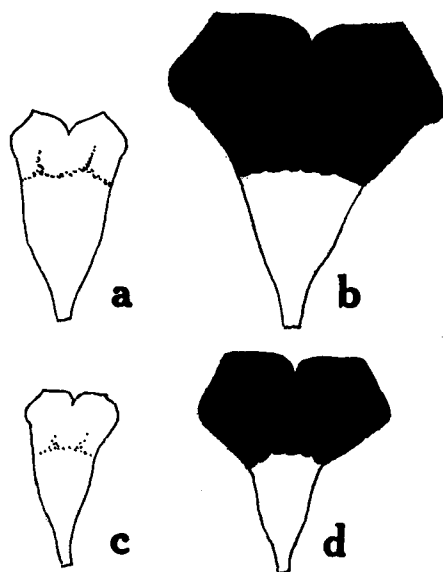

FIGURE 7.—a, b—two petal lobes and corresponding parts of the corolla tube of two flowers of a chimeral plant with large flowers (a—from a mosaic flower, b—from a self-purple flower); c, d—two petal lobes and corresponding parts of the corolla tube of two flowers of a chimeral plant with small flowers (c—from a mosaic flower, d—from a self-purple flower). Natural sizes.

rived from periblem, play also a part in the determination of flower sizes. This problem however will be discussed in another paper.

Figure 7c, d, represents the lobes and the tubes of two flowers taken from a chimeral individual. One of these flowers is self-colored (self-purple) while the other is mosaic. We see that the differences between them consist not only in the amount of pigment but also in the sizes of the lobes, the sizes of the corolla tube being the same in both flowers. The mutation which took place in this chimeral individual concerns both the sizes and the amount of pigment.

There are other chimeras in which the self-purple flowers are distinctly larger than in the above described individual. The lobes as well as the

tubes of such flowers are proportionally larger (figure 7a, b). The mosaic flowers of such chimeral plants are also larger (figure 7). It is possible that the difference between those two chimeral plants depends upon the genetical constitution of the periblem. The former chimeral individual had presumably the periblem determined by the "mosaic" allele while in the latter the periblem was possibly influenced by the "self-purple" allele. Both the smaller and the larger self-purple flowers would have on this hypothesis the same dermatogen but they would differ in the genetical constitution of the periblem. Anatomical investigations have shown that the epidermal cells of both types of self-purple flowers are of the same sizes.

THE NUMBER OF SEEDS AND THEIR GERMINATION

The plants with small mosaic flowers are smaller and weaker than those with large self-purple ones. They also have smaller fruits and a smaller number of seeds per fruit. In table 1 some data are given concerning the number of seeds per fruit in plants with large self-purple flowers and in those with small mosaic flowers.

TABLE 1

The number of seeds per fruit in two types of plants.

IN PLANTS WITH LARGE SELF-PURPLE FLOWERS			IN PLANTS WITH SMALL MOSAIC FLOWERS		
NO. OF PLANT	NUMBER OF FRUITS EXAMINED	AVERAGE NUMBER OF SEEDS PER FRUIT	NO. OF PLANTS	NUMBER OF FRUITS EXAMINED	AVERAGE NUMBER OF SEEDS PER FRUITS
A	7	171	G	9	60
B	20	143	H	24	139
C	11	149	K	16	101
D	6	260	M	13	64
F	25	127	P	12	70

The percent of germinating seeds was also greater in the plants with large self-purple flowers (table 2). I have chosen for germination tests the

TABLE 2

Seed germination.

PLANTS WITH LARGE SELF-PURPLE FLOWERS				PLANTS WITH SMALL MOSAIC FLOWERS			
NO. OF FRUIT	NUMBER OF SEEDS PER FRUIT	NUMBER OF SEEDS SOWN	NUMBER OF SEEDS GERMINATING	NO. OF FRUIT	NUMBER OF SEEDS PER FRUIT	NUMBER OF SEEDS SOWN	NUMBER OF SEEDS GERMINATING
A_1	160	144	128	H_{13}	175	144	116
A_2	195	144	134	H_{14}	175	144	112
A_3	175	144	129	H_{10}	170	144	98
A_4	150	144	123	H_{17}	175	144	105
A_5	185	144	130	H_{19}	165	144	91
A_7	148	144	122	H_{20}	160	144	100

largest fruits not differing notably as to the number of their seeds. The germination tests lasted 28 days in a temperature of 25°C during 6 hours a day and about 18°C during the remaining 18 hours.

<div align="center">THE POLLEN GRAINS</div>

In the garden varieties of *P. violacea* we find usually a large percent of defective pollen grains. The same is in our unstable race. We find in this race less than 50 percent of pollen grains well formed and apparently capable of further development; the remainder are deficient in size or content and are obviously non-functional.

The two extreme flower types differ in the percent of defective pollen; this percent is larger in small mosaic flowers than in self-purple ones. We found in typical flowers of the two extreme types of plants the following numbers: in the large self-purple flowers taken at random from different pollen sacs of 4 individuals 556 well-formed pollen grains and 775 abortive and in the small mosaic flowers from 5 plants 203 well-formed and 932 abortive pollen grains. In the first case we have to deal with 58 percent of abortive pollen and the second with 82 percent. The two extreme flower types do not differ significantly as far as the percentage of germination of their well-formed pollen grains is concerned. The germination test carried out by sowing the pollen grains in 10 percent cane sugar solution has proved however that the percent of germinated pollen grains is a little larger in the normal variety of Petunia than in our unstable race. The pollen grains are also larger in the large self-purple flowers than in the small mosaic ones.

<div align="center">. CYTOLOGICAL DATA</div>

The experimental data so far published by the writer and by Dr. M. SACHSOWA (formerly assistant of our Institute) and concerning the unstable race of *P. violacea* seemed to favor the hypothesis of unstable genes. However in view of great differences existing between the extreme flower types and involving so many characters it was necessary, before accepting or rejecting this theory, to examine the cytological nature of the unstable race. If there were cytological differences between the extreme types of flowers it would be difficult, of course, to accept the theory of unstable genes. In 1929 I entrusted to Miss A. SMÓLSKA the task of investigating this problem. These investigations published in 1932 were inadequate because they did not take into account the morphological characters of the chromosomes. Besides, the results of these researches could not account for all characteristic features of this race. I decided therefore to examine the problem anew and this time thanks to the work of DR. H. TELEZYŃSKI results were arrived at which show that the two extreme flower types do not differ from each other as far as the morphol-

ogy of chromosomes and their behavior are concerned. Dr. TELEŻYŃSKI did not confirm the observations of Miss SMÓLSKA as to the conjugation of non-homologous chromosomes.

All individuals of the unstable race which were examined by Dr. TELEŻYŃSKI differ from the normal varieties of *Petunia violacea* in being deprived of one satellite. In normal varieties the longest chromosome pair possesses two satellites. In the unstable race one of these satellites is missing. The pair of chromosomes in question is marked in figures 8 and 9 by A. In the chromosome Ab in which the satellite is missing the terminal

A Ab B B

FIGURE 8.—Somatic chromosome complement of the unstable race. The chromosomes which have been identified in mitosis and meiosis are marked by A and B. The chromosome Ab is without satellite. ×5200.

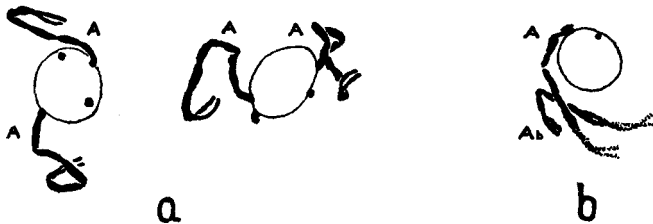a   b

FIGURE 9.—Satellited chromosome pair A in somatic prophase: a—in the normal variety of Petunia violacea both satellites are present (fixed in Navashin, stained by Feulgen's method). b—in the unstable race one satellite is missing (fixed in 2BD, stained by Feulgen's method). ×4500.

part of its shorter arm is homologous with the part of the shorter arm of the chromosome B, which may be inferred from the fact of the occurrence of triple terminal chiasmata observed several times by Dr. TELEŻYŃSKI between these chromosomes (figure 10).

These observations may be interpreted in the following way: During the evolution of the unstable race of *P. violacea* structural changes in the chromosomes have occurred consisting in the interchange of the terminal parts of the short arms of the chromosomes A and B. The chromosome behavior of the plants examined shows that those plants possess two chromosomes B which are identical, one chromosome A with satellite and one chromosome Ab with an exchanged part of the chromosome B (without satellite). Thus all plants of the unstable race examined are structural heterozygotes, trisomic on account of the interchanged part of the chro-

mosome B and monosomic in consequence of the deficiency of both the satellite and the terminal part of the chromosome A. Since the structural heterozygosity occurs in all plants of the unstable race we can draw the conclusion that in this race a balanced lethal mechanism is present. The zygotes deprived of both satellites are not viable because of the deficiency of the homologous parts in two chromosomes. The lack of plants with two satellites may be accounted for by the assumption that the lethal gene is located either in the satellite or in the terminal part of the chromosome A connected with this satellite.

The course of meiosis in the unstable race is normal and it is typical for the plants with complete terminalization of chiasmata. The mean chiasma
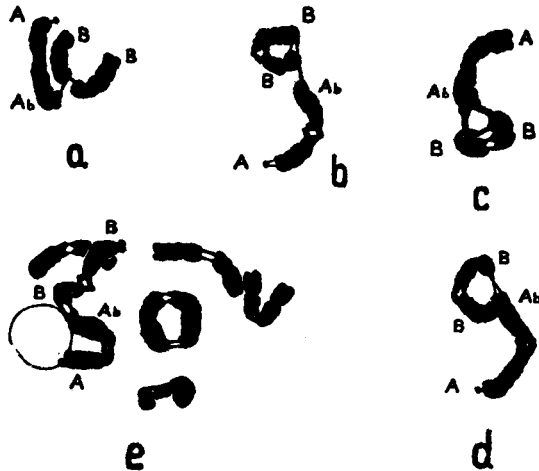

FIGURE 10.—Tetravalents in diakinesis in P.M.C. of the unstable race. In all figures the connections are seen between the chromosome Ab and one or two chromosomes of the pair B. a, b, c—tetravalents (separately represented) from different nuclei of the plant with large self-purple flowers (fixed in 2 BD, stained by Feulgen's method); d, e—tetravalents from the plant with small mosaic flowers (fixed in Bouin-Allen, stained by Feulgen's method); d—tetravalent with triple chiasma between the chromosome Ab and two chromosomes of the pair B; e—nucleus in diakinesis with 7 chromosome pairs (the tetravalent is lettered). ×4500.

frequency is approximately the same in plants of the unstable race and in those of the normal variety of *P. violacea* and the differences between particular plants are smaller than the ones between particular stamens of the same plant. The mean chiasma frequency per bivalent calculated for 1890 bivalents in 5 plants of the unstable race is 1.30. The mean number of ring bivalents per nucleus for 270 nuclei examined is 2.15, the modal value being 2. The two extreme flower types of the unstable race do not differ in this respect (table 3).

Abnormalities resulting from the association of homologous parts of the chromosomes A and B are very rare. More frequent are anomalies con-

TABLE 3

Frequency of the occurrence of ring bivalents in 270 nuclei.

FLOWER TYPE	NUMBER OF RING BIVALENTS						
	0	1	2	3	4	5	6
Small mosaic flowers	5	33	40	26	5	1	
Large self-purple flowers	5	30	63	45	13	3	1

sisting in the non-formation of chiasmata between the homologous chromosomes of one of these pairs. As a result we observe 2 univalents not associated in pairs in 2–5 percent of the pollen mother cells.

The most frequent cause of abnormalities is the phenomenon of lagging of the bivalents not completely terminalized in the anaphase I. These phenomena are apparently depending upon the external conditions, which may be inferred from the fact that the frequency of their occurrence is different in different pollen sacs of the same stamen. Such abnormalities are probably caused by the increased viscosity of the protoplasm. They occur also in normal varieties of Petunia and are probably the commonest cause of abortion of pollen grains in all varieties of *P. violacea*. The differences in the degree of pollen sterility between the two extreme flower types of the unstable race are possibly determined by the same mutable alleles which determine also the differences in sizes between the flowers. The above cytological characteristic of the unstable race is based entirely upon Dr. H. TELEŻYŃSKI's researches carried out in our Institute. The more detailed results of these researches will be published elsewhere in a separate paper.

SUMMARY

1. The extreme types of the unstable race of *Petunia violacea* differ in the following characters: (a) in the sizes of flowers, (b) in the distribution and in the amount of pigment in flowers, (c) in the sizes of the epidermal cells of petals, (d) in the percent of abortive pollen grains, and (e) in the percent of germinating seeds.

2. In small flowers the pigment is distributed in the form of small spots. These spots increase in sizes and usually fuse with one another in larger flowers. The larger spots are composed of dark purple cells surrounded by a layer of lighter purple cells. The smallest spots are composed entirely of lighter purple cells.

3. The epidermis and the marginal cells of the petals underlying the epidermis, derive from dermatogen. This fact accounts for the correlation existing between the sizes of petals and both the amount and the distribution of pigment. The genes acting in the dermatogen layer produce simul-

taneously the definite sizes of petals and the definite amount of pigment in the epidermis.

4. In spite of great differences existing between the extreme flower types of the unstable race these extreme types do not differ cytologically.

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